PRECIOUS METALS AND RHENIUM CONSORTIUM
Tetrachloroauric Acid Testing Programme

Secretariat: *Renaud Nicolay (EPMF)*

10 March 2015

CONSIDERATIONS ON THE NEED TO SUBMIT A TESTING PROPOSAL FOR THE IN VIVO MICRONUCLEUS ASSAY (OECD 474) ON TETRACHLOROAURIC ACID

LATEST MODIFICATIONS MADE IN THIS VERSION ARE HIGHLIGHTED IN TURQUOISE BLUE

1. Context

As part of the REACH integrated testing strategy with Tetrachloroauric Acid (CAS 16903-35-8, "TCA"), the Precious Metals and Rhenium Consortium ("PMC") sponsored an *In Vitro* Micronucleus Assay (OECD TG 487, "MN*vit*") on the substance.

The study returned a positive result, a conclusion not aligned on known/expected classification of the substance and experience on the workplace: no genotoxicity suspicion has been raised before.

In order to confront the validity of this result, i.e., obtain the substantial risk information needed, the relevant experts of PMC considered appropriate to sponsor an *In Vivo* Micronucleus Assay (OECD TG 474, "MN*viv*"), a consideration also mentioned in REACH Annex VIII column 2, item 8.4.

2. The Question

TCA is manufactured or imported in the tonnage band 10-100 tonnes/annum, hence submitted to information requirements laid down in Annexes VI, VII and VIII of the REACH regulation.

The MNviv assay is mentioned in Annexes VIII and IX. In Annex VIII column 2, item 8.4, the study is referred to in very general terms as an appropriate *in vivo* mutagenicity study which shall be considered, whilst in Annex IX it is referred to more precisely as an appropriate *in vivo* somatic cell genotoxicity study which shall be proposed by the registrant.

The different wording used in Annexes VIII and IX leads to some confusion and the following question: in complying with REACH for a substance manufactured/imported in the 10-100 tonnes/annum, can PMC assume that the decision as to whether or not to conduct the MNviv will be motivated by the information requirements laid down in the REACH Annex VIII and not Annex IX?

3. The Decision Process

The following table outlines the chronology that conducted to PMC's decision on the above question:

Table 1. Chronology of discussions held between PMC, external experts and authorities to answer the question under item 2 above.

Date	Event	Conclusion
Nov 2012	MLA study + FISH analysis at Covance results in positive clastogenic (chromosomal damage) properties of TCA	Need to discuss next steps of genotoxicity testing programme on TCA
Nov 2012	Review of above results by Dr David Kirkland	Confirms clastogenic properties of TCA
Nov 2012	Conference call between WCA, dedicated toxicology consultant, PMC, and David Kirkland to understand results and discuss about next steps to address suspicion of	Need to conduct an *In vivo* micronucleus study to confirm genotoxic effect of TCA. *In vivo* micronucleus study can be

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Date	Event	Conclusion
	genotoxicity of TCA	performed in combination or separately from OECD 422, with various pros and cons to be further considered
Jan 2013	Conference call between WCA and PMC to identify ideal test material and further elaborate on next steps	Recommend combined OECD 422 and *in vivo* micronucleus study OECD 474
Jul 2013	Experts conference call to agree on way forward	Recommend separate MTD/DRF+OECD 422 and *in vivo* micronucleus study mainly due to the fact that administered doses would not be the same for the OECD 422 and OECD 474
Aug 2013	Sample for MTD/DRF and OECD 422 tests are requested	MTD/DRF and OECD 422 to run first and inform in vivo micronucleus study parameters, as applicable
Sep 2013	Start of MTD/DRF test (phases 1 and 2 of OECD 422)	
Mar 2014	Launch of OECD 422 and discussion around tiered testing approach to bring TCA genotoxicity assessment to a conclusion	
Mar 2014	Opinion from UK REACH Helpdesk obtained, in summary: Where *in vitro* genotoxicity studies of low tonnage substances (Annex VII and VIII) give a positive result, follow-up testing is not mandated, but "shall be considered". This allows duty holders the option of "considering" the data they have available, and conducting further testing, if they regard it as necessary. For higher tonnage substances (Annex IX and X), the possibility of duty holder to "consider" has been removed and duty holders are required to submit a testing proposal for an appropriate *in vivo* test (if such data are not already available) (Full UK REACH Helpdesk response in Annex I of this document.)	At a dedicated conference call, the Au WG decided as follows: Because TCA is registered in a low tonnage band, Annex VIII applies and decision on whether or not to conduct an *in vivo* micronucleus test is with the registrants. It is PMC members' opinion that responsible product stewards will follow the option of proactive testing in order to fully assess the potential genotoxic effect of TCA and implement the associated classification, labelling, and risk management measures, if any, accordingly.
24 July 2014	Initiation of the PMC OECD TG 474 study with substance TCA	
04 Aug 2014	OECD 474 experiment start and is completed on 15 Aug 2014	
19 Aug 2014	The ECHA Chapter R.7a: Endpoint Specific Guidance (including mutagenicity information requirements) is updated on 19 August 2014 and published on the ECHA website. It requires that a testing proposal is submitted for any information requirement listed in Annex IX or X.	Studies under Annex IX or X now require that a testing proposal is submitted and approved by ECHA before the conduction of such a study. The OECD TG 474 experiment was completed by PMC before the release of the updated guidance.
Aug 2014	Preparation of this paper to report details behind opinion-forming	*In vivo* micronucleus test protocols at Covance can be signed and test can be launched

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4. Conclusion

Because TCA is registered in a low tonnage band, Annex VIII applies and decision on whether or not to conduct an *in vivo* micronucleus test is with the registrants. It is PMC members' opinion that responsible product stewards will follow the option of proactive testing, in order to fully assess the potential genotoxic effect of TCA without further delay, and implement any relevant classification, labelling, and risk management measures to protect the workers and users potentially exposed to TCA accordingly.

The In Vivo Micronucleus Assay on TCA is initiated on 27 July 2014 and the experimental phase completed on 15 Aug 2014. Therefore, the OECD TG 474 study was started and the in-life phase completed before the release on 19 Aug 2014, on the ECHA website, of the updated guidance specifying the obligation to submit and having approved by ECHA a testing proposal before conducting in vivo tests in REACH Annex IX and X.

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Annex I

1. UK REACH Response

From: Ian.Indans@hse.gsi.gov.uk [mailto:Ian.Indans@hse.gsi.gov.uk] **On Behalf Of** UKREACHCA@hse.gsi.gov.uk
Sent: mercredi 12 mars 2014 11:50
To: Owen Green
Subject: REACH - Annex VIII and Annex IX Genetoxicity Testing - Helpdesk reference - 1003IRI14-2408

Dear Owen

Generally, where *in vitro* genotoxicity tests give a positive result, regulatory schemes require appropriate follow up to determine whether the substance is an *in vivo* genotoxicant, and potentially subject to stringent regulatory control. The REACH Regulation information Annexes follow the same testing paradigm, but introduced some tonnage-related aspects too.

Where *in vitro* genotoxicity tests of low tonnage substances give a positive result, (Annex VII and VIII),, follow-up testing is not mandated, but "shall be considered" In our opinion this allows duty holders the option of "considering" the data they have available, and conducting further testing,if they regard it as necessary. At Annex IX, this possibility has been removed and duty holders are required to submit a testing proposal for an appropriate *in vivo* test, if such data are not already available. .

One of the features of REACH is that it has placed much more responsibility in the hands of the manufacturers/importers. The option to conduct *in vivo* follow up at Annex VII and VIII could be seen in this context, allowing duty holders the choice whether to pursue *in vivo* genotoxicity testing. It is likely that responsible product stewards will follow the option of earlier testing.

In our opinion, where *in vivo* follow-up studies are conducted at Annex VII or VIII they should be considered as Annex VII or VIII studies and a testing proposal does not appear to be required.

I hope this helps

Kind regards

Ian

Ian Indans
REACH and CLP Helpdesk
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From: Owen Green [mailto:Owen.Green@wca-environment.com]
Sent: 10 March 2014 14:05
To: UKREACHCA
Subject: Re: REACH Annex VIII vs Annex IX Genetox tests Ian

In the case of a substance yielding a positive result in an Annex VIII in vitro micronucleus test should the ensuing in vivo mutagenicity tests considered to be an Annex VIII or an Annex IX test?

For ease of reference, the specific comments in the relevant regulations are as follows:

Annex VIII, Col 2:

Appropriate in vivo mutagenicity studies shall be **considered** in case of a positive result in any of the
genotoxicity studies in Annex VII or VIII.

Annex IX, Col 2:

If there is a positive result in any of the in vitro genotoxicity studies in Annex VII or VIII and there are
no results available from an in vivo study already, an appropriate in vivo somatic cell genotoxicity study
shall be proposed by the registrant.
If there is a positive result from an in vivo somatic cell study available, the potential for germ cell muta-
genicity should be considered on the basis of all available data, including toxicokinetic evidence. If no
clear conclusions about germ cell mutagenicity can be made, additional investigations shall be consid-
ered.

My personal view is that if there is a positive in vitro study under Annex VIII, the legislation can be interpreted so that there is a duty of care/moral position to define whether there is a risk to humans from exposure to the substance, ie by appropriate in vivo studies. Therefore such studies should be conducted without waiting years for the registration and test proposals to be considered by ECHA. Is this the way that these sections should be interpreted?

Very best regards,

Owen Green

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